DATA EVALUATION RECORD

PICOXYSTROBIN (ZA1963)

Study Type: OPPTS 870.3100 [§82-1a], Subchronic Oral Toxicity Study in Mice

Work Assignment No. 7-1-256 B (MRID 48073732)

Prepared for
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DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity [feeding]-[mouse]; OPPTS 870.3100 [§82-1a] (rodent); OECD 408.

PC CODE: 129200 **DP BARCODE:** D378236
TXR #: 0056696 **SUBMISSION #:** S873059

TEST MATERIAL (PURITY): Picoxystrobin (99% a.i.)

SYNONYMS: ZA1963; methyl (α*E*)-α-(methoxymethylene)-2-[[[6-(trifluoromethyl)-2-pyridinyl]oxy]methyl]benzeneacetate

CITATION: Rattray, N.J. (1996) ZA1963: 90 day feeding study in mice. Central Toxicology Laboratory, Macclesfield, Cheshire, UK. Laboratory Study No.: CTL/T/2915, June 26, 1996. MRID 48073732. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company, Wilmington, DE

EXECUTIVE SUMMARY: In a subchronic oral toxicity study (MRID 48073732), picoxystrobin (ZA1963; 99% a.i.; Batch No. P13) was administered in the diet to C57BL/10J$_f$AP/Alpk mice (10/sex/dose) at doses of 0, 200, 800, 1600, or 2400 ppm (equivalent to 0/0, 33.2/43.8, 137.3/176.1, 290.8/358.5, and 421.6/534.8 mg/kg/day for males/females) for 13 weeks.

No adverse, treatment-related effects were observed on mortality, clinical signs, food consumption, organ weights, or gross or microscopic pathology.

Body weights were decreased ($p<=0.05$; except as noted) as follows: (i) sporadically throughout the study in the 800 ppm males (not statistically significant [NS]) and in the females (decr 3-7%); (ii) sporadically throughout the study in the 1600 ppm males (decr 2-7%) and females (decr 3-5%); and (iii) in the 2400 ppm males sporadically throughout the study (decr 2-6%) and females often throughout the study (decr 4-10%). At 800 ppm and above, body weight gains were decreased in males and in females during the initial week, contributing to decreased overall (Days 1-92) body weight gains of 10-28% in males and 18-34% in females.

At 1600 and 2400 ppm, food utilization (g food/100 g body weight gain) was decreased ($p<=0.05$; except as noted) during Days 1-28 in males by 38-52% and in females by 15-44%. The initial effects on food utilization in 1600 and 2400 ppm groups contributed to an overall

(Days 1-91) decrease of 25-32% in males and 10-25% in females. At 800 ppm, a slight decrease in food utilization was also found demonstrating a threshold treatment-related effect.

The LOAEL is 800 ppm (137.3 mg/kg/day) based on decreases in body weights , body weight gains and food utilization. The NOAEL is 200 ppm (33.2 mg/kg/day).

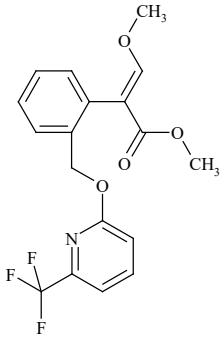
This study is classified as **unacceptable/non-guideline** and does not satisfy the guideline requirements (OPPTS 870.3100; OECD 408) for a subchronic oral toxicity study in the mouse. Hematology and clinical chemistry were not performed. Numerous organs were not weighed, and numerous tissues were not evaluated microscopically. However, this study provides useful data for dose-selection for longer term studies.

COMPLIANCE: A signed and dated Data Confidentiality statement was provided. A GLP Compliance statement was provided, but did not state whether this study was in compliance with 40 CFR Part 160 and 40 CFR Part 172. No Quality Assurance statement was provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Picoxystrobin

Description:	White solid
Batch No.:	P13
Purity (w/w):	99% a.i.
Stability of compound:	The test compound was stable in the dietary formulations for at least 78 days at room temperature.
CAS #:	117428-22-5
Structure:	

2. Vehicle: Diet

3. Test animals

Species:	Mouse
Strain:	C57BL/10J_fAP/Alpk
Age and group mean weight at study initiation:	Approximately 5 weeks old; 21.9-22.2 g males; 17.4-17.8 g females
Source:	Rodent Breeding Unit, Zeneca Pharmaceuticals, Alderley Park, England
Housing:	5 per cage (same sex) in multiple mouse racks
Diet:	CT1 diet (Special Diets Services Limited, Stepfield, Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions	
Temperature:	21±2ºC
Humidity:	40-70%
Air changes:	At least 15/hour
Photoperiod:	12 Hours light/12 hours dark
Acclimation period:	At least 5 days

B. STUDY DESIGN

1. In life dates: Start: August 8, 1995; End: November 10, 1995

2. Animal assignment/dose levels: The animals were randomly assigned to the test groups shown in Table 1.

TABLE 1: Study design [a]			
Test group	**Dose to animal (ppm)**	**Compound intake (mg/kg/day in M/F)**	**No. mice/sex killed at Week 13**
Control	0	0/0	10
Low	200	33.2/43.8	10
Mid	800	137.3/176.1	10
Mid-high	1600	290.8/358.5	10
High	2400	421.6/534.8	10

a Data were obtained from pages 13, 69, and 70 of MRID 48073732.

3. **Dose-selection rationale:** The Sponsor stated that the dose levels selected for this study were based on the results of a 28-day feeding study in the mouse carried out in this laboratory.

4. **Dose preparation and analysis:** The test compound was mixed with the diet to form a pre-mix, which was further diluted with appropriate amounts of the diet to achieve the desired concentrations. The frequency of preparation was not reported. Test compound stability in the diet was evaluated in the 200 and 2400 ppm dietary formulation for up to 78 days at room temperature. Homogeneity (top, middle, and bottom strata) was also evaluated in the 200 and 2400 ppm dietary formulations. Concentrations were measured twice during the study for each dietary concentration.

Results

Homogeneity (% coefficient of variation): 0.30-2.54%

Stability (% of initial): 93.3-100.0%

Concentration (% of nominal): 87.5-103.4%

The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the animals was acceptable.

5. **Statistics:** All data were evaluated using the GLM procedure in SAS, separately for males and females. The following statistical analyses were performed.

PARAMETER	STATISTICAL ANALYSES
Body weight	Body weights were considered by analysis of covariance on initial (week 1) body weight. [a]
Food consumption Food utilization	Analysis of variance was conducted. [a]
Organ weights	Analysis of variance and analysis of covariance on final body weight were performed. [a]

a Analyses of variance and covariance allowed for the replicate structure of the study design. Least-squares means for each group were calculated using the LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least squares mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least squares mean using a two-sided Student's t-test, based on the error mean square in the analysis.

Statistical analysis was performed on food consumption and utilization where n=2. Although this is not appropriate, generally the statistical analyses conducted in this study was considered appropriate.

C. METHODS

1. Observations

a. Cageside observations: All animals were checked daily for changes in clinical condition or behavior.

b. Clinical examinations: Detailed clinical observations were performed weekly.

c. Neurological evaluations: Neurological evaluations were not conducted in this study; however, acute and subchronic neurotoxicity studies in rat were concurrently submitted (MRIDs 48073753 and 48073752, respectively).

2. Body weight and body weight gain: All animals were weighed prior to treatment, weekly until scheduled termination, and at necropsy.

3. Food consumption, food utilization, and compound intake: Food consumption (g/mouse/day) was measured for each cage and was reported daily for the initial week and weekly thereafter beginning with Week 2. Food utilization per cage (g body weight gain/100 g food) was calculated for Days 1-28, 29-56, 57-91, and 1-91. Compound intake (mg/kg/day) was calculated from the group mean bodyweight and food consumption data, and overall compound intake was reported (Table 1).

4. Ophthalmoscopic examination: The eyes were not examined.

5. Hematology and clinical chemistry: Hematology and clinical chemistry were not performed.

6. Urinalysis: Urinalysis was not performed, but is optional under Guideline 870.3100.

7. Sacrifice and pathology: Animals were euthanized by exsanguinations under terminal anesthesia induced by halothane Ph. Eur. vapor. The following CHECKED (X) tissues were collected; however, only a few of the tissues were examined microscopically (specified below). Additionally, the (XX) organs were weighed, paired organs weighed together.

	DIGESTIVE SYSTEM
	Tongue
X	Salivary glands*
X	Esophagus*
X	Stomach*
X	Duodenum*
X	Jejunum*
X	Ileum*
X	Cecum*
X	Colon*
X	Rectum*
XX	Liver*+
X	Gall bladder (not rat)*
	Bile duct (rat)
X	Pancreas*
	RESPIRATORY
X	Trachea*
X	Lung*
X	Nose*
X	Pharynx*
X	Larynx*

	CARDIOVASC./HEMAT.
X	Aorta*
X	Heart*+
X	Bone marrow*
X	Lymph nodes*
X	Spleen*+
X	Thymus*+
	UROGENITAL
XX	Kidneys*+
X	Urinary bladder*
XX	Testes*+
X	Epididymides*+
X	Prostate*
X	Seminal vesicles*
X	Ovaries*+
X	Uterus*+
X	Mammary gland* (females)
X	Cervix

	NEUROLOGIC
XX	Brain*+
X	Peripheral nerve (sciatic)*
X	Spinal cord*
X	Pituitary*
X	Eyes (retina, optic nerve)*
	GLANDULAR
XX	Adrenal gland*+
X	Lacrimal/Harderian gland
X	Parathyroid*
X	Thyroid*
	OTHER
X	Bone (femur and sternum)
X	Skeletal muscle
X	Skin*
X	Knee joint
X	All gross lesions and masses*

* Recommended for 90-day oral rodent studies based on Guideline 870.3100
\+ Organ weights required for rodent studies.

The Sponsor stated that all tissues were fixed in an appropriate fixative. The following tissues from the control and 2400 ppm groups were routinely processed, stained with hematoxylin and eosin, and examined microscopically:

abnormal tissue	gall bladder	spinal cord
adrenal gland [a]	kidney [a]	spleen [a]
brain [a]	liver [a]	testis [a]
epididymis	ovary	trachea

a Although the Methods stated all these tissues were examined histologically, only results from the designated tissues were presented in the tabulated data (Table 11 in the study report).

II. RESULTS

A. OBSERVATIONS

1. **Mortality:** No mortality was observed.

2. **Clinical signs of toxicity:** No treatment-related effect was noted on clinical signs. Hair loss was increased in the 1600 (4 affected) and 2400 (3 affected) ppm males compared to 0 controls, but this effect was considered incidental.

B. BODY WEIGHT AND BODY WEIGHT GAIN:

Body weights and body weight gains were decreased in the 800 ppm and above groups (Table 2). Body weights were decreased ($p \leq 0.05$; except as noted) as follows: (i) sporadically throughout the study in the 800 ppm males (not statistically significant [NS]) and in the females (↓3-7%); (ii) sporadically throughout the study in the 1600 ppm males (↓2-7%) and females (↓3-5%); and (iii) in the 2400 ppm males sporadically throughout the study (↓2-6%) and females often throughout the study (↓4-10%). At 800 ppm and above, body weight gains were decreased in males and during the initial week, contributing to decreased overall (Days 1-92) body weight gains in males and females. At 200 ppm, no treatment-related effect on body weights and body weight gains was observed.

TABLE 2. Body weights and body weight gains (g) in mice treated with Picoxystrobin in the diet for 13 weeks [a]

Day(s)	Dose (ppm)				
	0	200	800	1600	2400
Males					
1 [b]	22.0±1.4	21.9±1.5	22.1±1.6	22.2±1.6	21.9±1.6
3 [c]	22.0	22.2	21.8	21.3** (↓3)	20.7** (↓6)
4 [c]	22.5	22.3	22.1	22.0* (↓2)	21.6** (↓4)
7 [c]	22.9	23.4* (↑2)	22.8	22.5	22.4* (↓2)
29 [c]	25.4	26.2	24.9	23.7* (↓7)	24.0
92 [c]	29.2	30.5	28.6	27.2* (↓7)	27.4* (↓6)
Days 1-7 [d]	0.9	1.4	0.7 (↓22)	0.5 (↓44)	0.4 (↓56)
Days 7-43	3.7	4.0	3.4 (↓8)	2.2 (↓41)	2.5 (↓32)
Days 43-92	2.6	3.1	2.4 (↓8)	2.5 (↓4)	2.5 (↓4)
Days 1-92	7.2	8.5	6.5 (↓10)	5.2 (↓28)	5.4 (↓25)
Females					
1 [b]	17.5±1.4	17.8±0.9	17.4±1.2	17.6±0.8	17.6±1.3
2 [c]	18.1	17.8	17.6* (↓3)	17.3** (↓4)	17.0** (↓6)
3 [c]	18.2	18.3	17.9	17.7* (↓3)	17.1** (↓6)
4 [c]	18.2	18.2	17.9	17.7* (↓3)	17.5** (↓4)
29 [c]	22.4	22.2	21.5* (↓4)	21.6* (↓4)	20.2** (↓10)
36 [c]	22.9	22.6	21.4** (↓7)	21.8* (↓5)	21.0** (↓8)
92 [c]	25.3	25.2	23.8** (↓6)	24.3* (↓4)	22.9** (↓9)
Days 1-7 [d]	1.2	1.1	0.5 (↓58)	0.6 (↓50)	0.7 (↓42)
Days 7-43	4.3	4.0	3.8 (↓12)	3.8 (↓12)	2.9 (↓33)
Days 43-92	2.5	2.4	1.9 (↓24)	2.2 (↓12)	1.7 (↓32)
Days 1-92	8.0	7.5	6.2 (↓23)	6.6 (↓18)	5.3 (↓34)

a Data (n=10) were obtained from Table 6 on pages 40-45 in MRID 48073732. Percent difference from controls is included in parentheses, and was calculated by the reviewers.

b Mean weights ± SD

c Adjusted (covariable is Week 1) mean weights

d Body weight gains were calculated by reviewers from mean body weights reported in the cited data. Statistical analyses

were not performed.
* Significantly different (p≤0.05) from the control groups
** Significantly different (p≤0.01) from the control groups

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. **Food consumption:** For the overall treatment period (Weeks 1-13), no adverse effect was observed on food consumption in any group. Food consumption was transiently decreased (p≤0.05) on Day 1 in the 800 ppm and above males (↓22-51%) and females (↓17-33%). Other differences in food consumption in the male groups were unrelated to dose. Decreases (p≤0.05) in food consumption were sporadic and slight in the female groups after Day 1.

2. **Food utilization:** At 1600 and 2400 ppm, food utilization (g food/100 g body weight gain) was decreased (p≤0.05; except as noted) during Days 1-28 in males by 38-52% and in females by 15-44% (Table 3). These initial effects on food utilization contributed to an overall (Days 1-91) decrease of 25-32% in males and 10-25% in females (NS in the 1600 ppm females). At 800 ppm, a slight decrease in food utilization was seen, but this decrease did not attain statistical significance. An incidental increase was noted in the overall food utilization of the 200 ppm males. All other food utilization values in the treated groups were similar to controls.

TABLE 3. Food utilization (g food/100 g bwg) in mice treated with Picoxystrobin in the diet for 13 weeks [a]

Days	Dose (ppm)				
	0	200	800	1600	2400
Males					
Days 1-28	2.90±0.74	3.70±0.18	2.51±0.08	1.38±0.12* (↓52)	1.80±0.34* (↓38)
Days 1-91	1.88±0.02	2.24±0.03* (↑19)	1.67±0.15	1.28±0.10** (↓32)	1.41±0.02** (↓25)
Females					
Days 1-28	3.62±0.35	3.55±0.44	3.17±0.20	3.07±0.69* (↓15)	2.04±0.36** (↓44)
Days 1-91	1.66±0.11	1.69±0.12	1.44±0.06	1.50±0.01 (↓10)	1.24±0.12** (↓25)

a Data (n=2) were obtained from Table 8 on page 50 in MRID 48073732. Percent difference from controls is included in parentheses, and was calculated by the reviewers.
* Significantly different (p≤0.05) from the control groups
** Significantly different (p≤0.01) from the control groups

3. **Compound consumption:** Compound intake values (mg/kg/day) are presented in Table 1 of this DER.

D. SACRIFICE AND PATHOLOGY

1. **Organ weight:** No adverse, treatment-related effects were noted on organ weights. Only minor differences (p≤0.05) were observed that were not corroborated by histology.

2. **Gross pathology:** No treatment-related effect was observed during necropsy.

3. **Microscopic pathology:** No adverse, treatment-related effect was observed during histology. Increased incidences of minimal hepatocyte hypertrophy were noted in the 1600

and 2400 ppm males and the 800 ppm and above females. This finding was not considered adverse due to the low severity and was considered an adaptive effect.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATOR'S CONCLUSIONS: There were reductions in food consumption and body weight, compared to concurrent control animals, and increases in liver weight, after adjustment for terminal bodyweight, in animals at dose levels of 2400, 1600 or 800 ppm ZA1963. Food utilization was less efficient in animals given 2400 or 1600 ppm ZA1963. There was a treatment related increase in the incidence of minimal hepatocyte hypertrophy in the livers of males given 2400 or 1600 ppm ZA1963 and in livers of females given 2400, 1600 or 800 ppm ZAl963. The LOAEL is 800 ppm, and the NOAEL is 200 ppm.

B. REVIEWER'S COMMENTS: No adverse, treatment-related effects were observed on mortality, clinical signs, food consumption, organ weights, or gross or microscopic pathology.

Body weights were decreased ($p \leq 0.05$; except as noted) as follows: (i) sporadically throughout the study in the 800 ppm males (not statistically significant [NS]) and in the females (↓3-7%); (ii) sporadically throughout the study in the 1600 ppm males (↓2-7%) and females (↓3-5%); and (iii) in the 2400 ppm males sporadically throughout the study (↓2-6%) and females often throughout the study (↓4-10%). At 800 ppm and above, body weight gains were decreased in males and females during the initial week, contributing to decreased overall (Days 1-92) body weight gains in males and females.

At 1600 and 2400 ppm, food utilization (g food/100 g body weight gain) was decreased ($p \leq 0.05$; except as noted) during Days 1-28 in males by 38-52% and in females by 15-44%. . These initial effects on food utilization in 1600 and 2400 ppm groups, contributed to an overall (Days 1-91) decrease of 25-32% in males and 10-25% in females (NS in the 1600 ppm females). At 800 ppm, a slight decrease in food utilization was seen. Although this decrease did not attain a statistical significance, the next two higher dose levels demonstrated more severe decrease in food utilization making the effect seen in 800 ppm a border threshold treatment-related effect.

The reviewers disagree with the Sponsor on the following points: For the overall treatment period (Weeks 1-13), no adverse effect was observed on food consumption in any group. Food consumption was transiently decreased ($p \leq 0.05$) on Day 1 in the 800 ppm and above males (↓22-51%) and females (↓17-33%), possibly due to a lack of palatability. Other differences in food consumption in the male groups were unrelated to dose. Decreases ($p \leq 0.05$) in food consumption were sporadic and slight in the female groups after Day 1. Therefore, the reviewers conclude that an adverse effect on food consumption was not observed.

Slightly increased liver weights, accompanied by minimal hepatocyte hypertrophy, were observed. In the absence of corroborating evidence of hepatotoxicity, these effects were considered adaptive and not adverse. However, the decrease in body weights, body weight

gains and food utilization were considered treatment related because the mode of action for picoxystrobin was inhibition of electron transport system in mitochondria.

The LOAEL is 800 ppm (137.3 mg/kg/day) based on decreased body weights , body weight gains and slight decrease in food utilization which was more severe at the next higher tested dose levels. The NOAEL is 200 ppm (equivalent to 33.2 mg/kg/day).

This study is classified as **unacceptable/non-guideline** and does not satisfy the guideline requirements (OPPTS 870.3100; OECD 408) for a subchronic oral toxicity study in the mouse. Hematology and clinical chemistry were not performed. Numerous organs were not weighed, and numerous tissues were not evaluated microscopically. However, this study provides useful data for dose-selection for longer term studies.

C. **STUDY DEFICIENCIES:** The Sponsor did not state that this study was to be considered non-guideline; however, major departures from the guidelines were noted. The following deficiencies were observed:

- Clinical chemistry was not performed.
- Hematology was not performed.
- Numerous organs were not weighed, despite guideline recommendations.
- Microscopic evaluation was not performed on numerous tissues recommended by the guideline.
- At least 3 cages of animals should have been used in order to allow appropriate statistical analysis of food consumption and food efficiency.